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CHEMOSYNTHETIC GAS EXCHANGER

Second Quarterly Progress Report
for period ending December 18, 1963

ER-13270-2

[redacted]

[2] (NASA Contract NASw-713)

Investigator: Leonard Bongers [1963] 14p +fa

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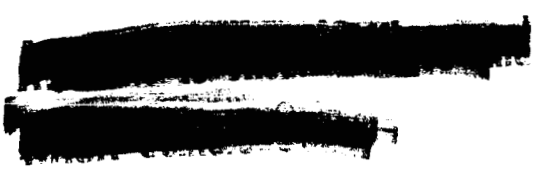
FOREWORD

This report has been prepared by the Research Department of the Martin Company in compliance with NASw-713.



INTRODUCTION

This second quarterly report describes research conducted under Contract NASw-713 in the period September 3, 1963 through December 17, 1963. The work on which is reported is concerned with the effect of oxygen upon growth rate, conversion efficiency, and cell composition. Also the effect of N - limited growth will be discussed. Scheduled work for the next reporting period will be indicated.

A. Effect of O_2 Upon Growth, Efficiency and Composition

An earlier report[1] indicated that the optimal oxygen concentration for cell proliferation was on the order of 0.010 mmole O_2 per liter, a concentration in equilibrium with 10% O_2 in the gas phase at atmospheric pressure.

In continuation of these experiments further investigations were conducted dealing with the effect of oxygen concentration upon the rate of gas uptake, efficiency of conversion and the relationship between oxygen supply and cell composition.

The three oxygen concentrations investigated were 10%, 20% and 30% O_2. Rates of gas uptake were measured with a Macro-respirometer-- 20 to 50 ml suspension-volume--and with the conventional Warburg technique. In case the Warburg technique was used to determine metabolic rates, very dilute suspensions were employed (2 to 3 ml), and precautions were taken that gas equilibrium existed between fluid and gaseous phase. All metabolic and growth rates were measured at 35°C.

Table 1 represents a series of measurements of the effect of oxygen concentration on conversion efficiency. Results in Table 1 illustrate that with higher oxygen concentrations the number (expressed as N) of hydrogen oxidations per mole of carbon dioxide incorporated in new cell material increases. Two conclusions can be drawn from these results. First, the overall growth efficiency becomes less at relatively high oxygen concentrations (15 and 20%). Second, the presence of relatively high oxygen concentrations has a profound effect on the specific rate (Q_{CO_2}) of carbon dioxide uptake. The relatively low efficiency reflects a higher power demand, while the diminished specific rate would result either in an increased volume requirement or in the use of relatively more concentrated cell suspensions.

Table 1

Effect of Oxygen on Growth Efficiency and the Power Requirement of a Chemosynthetically Balanced Ecosystem

Gas phase	70% H_2	10% CO_2	O_2 as indicated	N_2 balance
O_2%	5	10	15	20
N	3.9 ± 0.3	4.2 ± 0.2	5.7 ± 0.3	6.0 ± 0.4
Q_{CO_2}	183 ± 9	185 ± 4	154 ± 9	139 ± 7
Watts * cont	700	740	920	900

* Calculations based on an electrolytical efficiency of 80%

The same inhibiting effect of oxygen is borne out in the results plotted-- Fig. 1 A, B. At 10% oxygen, the rate of gas uptake per unit volume of suspension increases steadily up to a cell concentration reached after about 3 hours of growth (Fig. 1 A). At 20% O_2 only a slight increase occurs, while at 30% O_2, the gas uptake initially decreases and then becomes constant. The specific rates, as plotted in Fig. 1 B, present a similar phenomenon; with 30% O_2 in the gas phase a considerable oxygen inhibition occurs. The gradually diminishing specific rate, as observed with 10% O_2 in the gas phase, is due to the gas limiting situation which developes as a result of the higher oxygen demand. It is difficult to distinguish clearly between decrease in specific rate due to oxygen inhibition and the decrease due to oxygen limitation but for the shape of the curves and, as discussed later, the composition of the material formed under these conditions.

Gas limitation leads, according to Schlegel et al[2] to the formation of fat inclusions in hydrogen bacteria. In order to verify the effect of oxygen upon the formation of fat inclusions, the cells obtained from experiments as described in Fig. 1 were analyzed by a procedure adapted from Williamson et al [3]. The results of these analyses are illustrated in Fig. 2. As shown, no fat inclusions are formed in cells cultivated at 30% O_2, while at 10% O_2 a considerable fat formation occurs. These results are also borne out by the electron micrograph of Fig. 3, a "lean" dividing cell, and of Fig. 4, in which the cells show distinct globular inclusions, in some sharply distinguisable in the cytoplasmatic material.

In Fig. 5, a time course experiment conducted with 10% O_2 in the gaseous atmosphere , the relationship between fat formation (T-incl.) and the rate of gas uptake (G.U.) is demonstrated. After 2, 5, to 3 hours, with the onset of the formation of, - apparently synthetically inactive-, fatty storage material, the rate of gas uptake becomes constant and finally declines. The same "breaking point" can be observed in Fig. 1 B, with respect to the specific rate of gas uptake with 10% O_2 in the gas phase.

The linear increase with time of cell material (T-cell) indicates that the rate of carbon dioxide assimilation, calculated per unit volume, is not affected. As no cell division is obvious from the electron micrographs, one would expect that rather cell enlarging than cell division occurs. No precise determination of the efficiency involved in the formation of this lipoid material can be made from these experiments. Efforts to determine this aspect of Hydrogenomonas cultivation are presently underway.

B. N-Limited Growth

Growth of hydrogen bacteria in N - limited nutrient media leads to the formation of cell material with a high ratio of lipoid inclusions. Nitrogen starvation strongly decreases however the specific rate and also the rate of gas uptake, calculated per unit volume of suspension (see Table 2). When normal, "lean" cells are resuspended in a nitrogen free medium, the gas uptake decreases to about three quarters of the starting value upon which a constant level is maintained.

Table 2

Gas Consumption Under N - Limiting Conditions
Gaseous Substrate: 80% H_2; 10% O_2; 10% CO_2
Temperature 35°C; Hydrogenomonas eutropha

Time (min.)	Gas Uptake l/hr		HyOx fraction
	Vol (l)	d.w (g)	
0			
30	3.50		
60	3.40	1.5	.65
90	3.50		
120	3.40	1.4	.68
150	3.40		
180	3.20	1.2	.66
210	3.10		
240	3.00	1.0	.67
270	2.80		
300	2.60	0.9	.77

After 3 to 4 hrs of N-starvation the rate decreases further. The efficiency of conversion, however, is only slightly affected as indicated by the number of hydrogen oxidation (HyOx-fraction, Table 2). It seems, therefore, that fat formation, at least under conditions of N-starvation, occurs at an efficiency level comparable to regular cell growth and multiplication.

In Fig. 6, the time course of cell mass increase and the increase in the density of inclusions is illustrated. Fat formation is initiated immediately in the absence of a usable nitrogen source, and the characteristics of the curves in Fig. 6 indicate that most of the metabolic activity is geared to the formation of lipoid material.

Electron micrographs of these "fat" cells (Fig. 7) show that most of the cytoplasmatic material is composed of these inclusions, while very little nuclear material can be seen.

Table 3

Cell "Fattening" in Presence (I) and Absence (II) of Urea
Temperature: 35°C; Gaseous Substrate: 75% H_2, 15% O_2, 10% CO_2;
Hydrogenomonas eutropha

Time		0 hrs	38 hrs
Dry weight g/l	I	1.90	8.5
	II	1.90	6.7
Nitrogen content %	I	1.90	7.4
	II	1.90	4.1
Fat Fraction	I	0.03	0.22
	II	0.03	0.43

Table 3 shows the results of a preliminary analysis of hydrogen bacteria cultivated in the absence of nitrogen (II) and under conditions which induce fat formation in the presence of nitrogen (I). The nitrogen content of N-starved cells decreased to 4%, while the fat ratio increased to 0.43. Further analyses are required to determine the actual fat content of these cells. This work is scheduled presently.

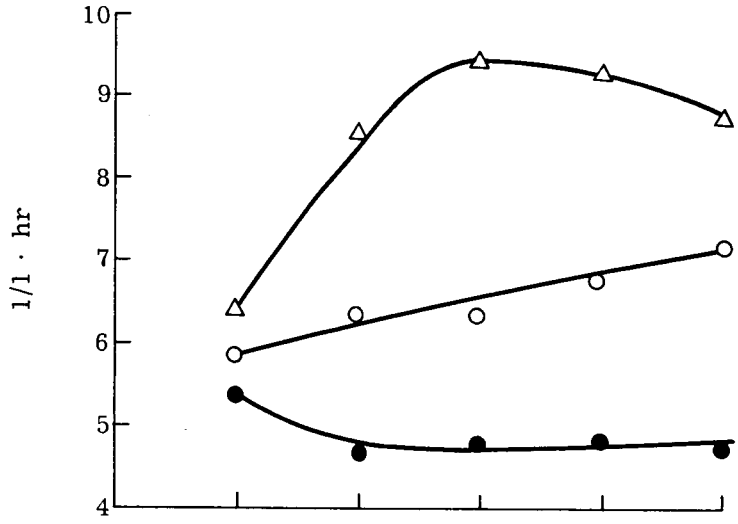
References

1. Bongers, L., "Chemosynthetic Gas Exchanger", Martin Company RM-153; May 1963

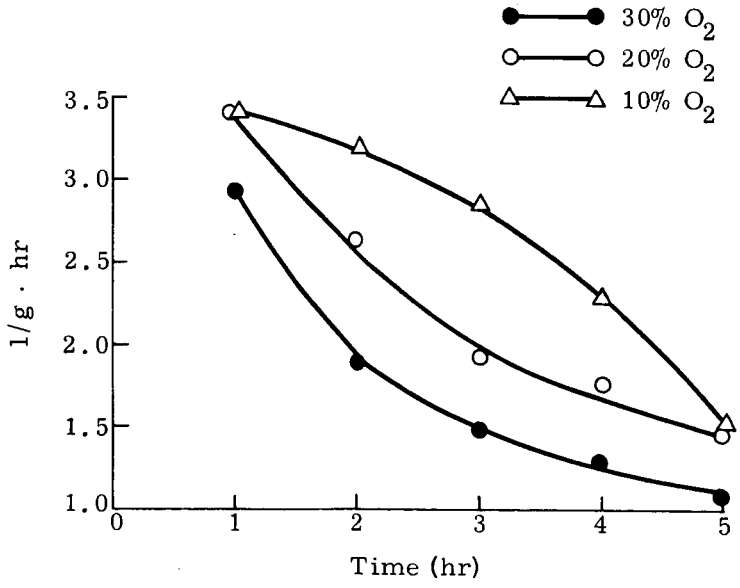
2. Schlegel, H. G., et al, Nature, 191; 463-465; 1961

3. Williamson, D. H., et al, J. Gen. Microbiol. 19; 198-209, 1958

Starting dry weight: 2 grams per liter approximately



A: Uptake of $H_2 + O_2 + CO_2$ per liter of suspension per hour



B: Specific rate of uptake expressed in liters per gram dry weight, per hour

Fig. 1. Gas Uptake by Hydrogenomonas Eutropha, With 10, 20, 30% O_2, 60% H_2, and 10% CO_2 in Gas Phase (N_2 balance)

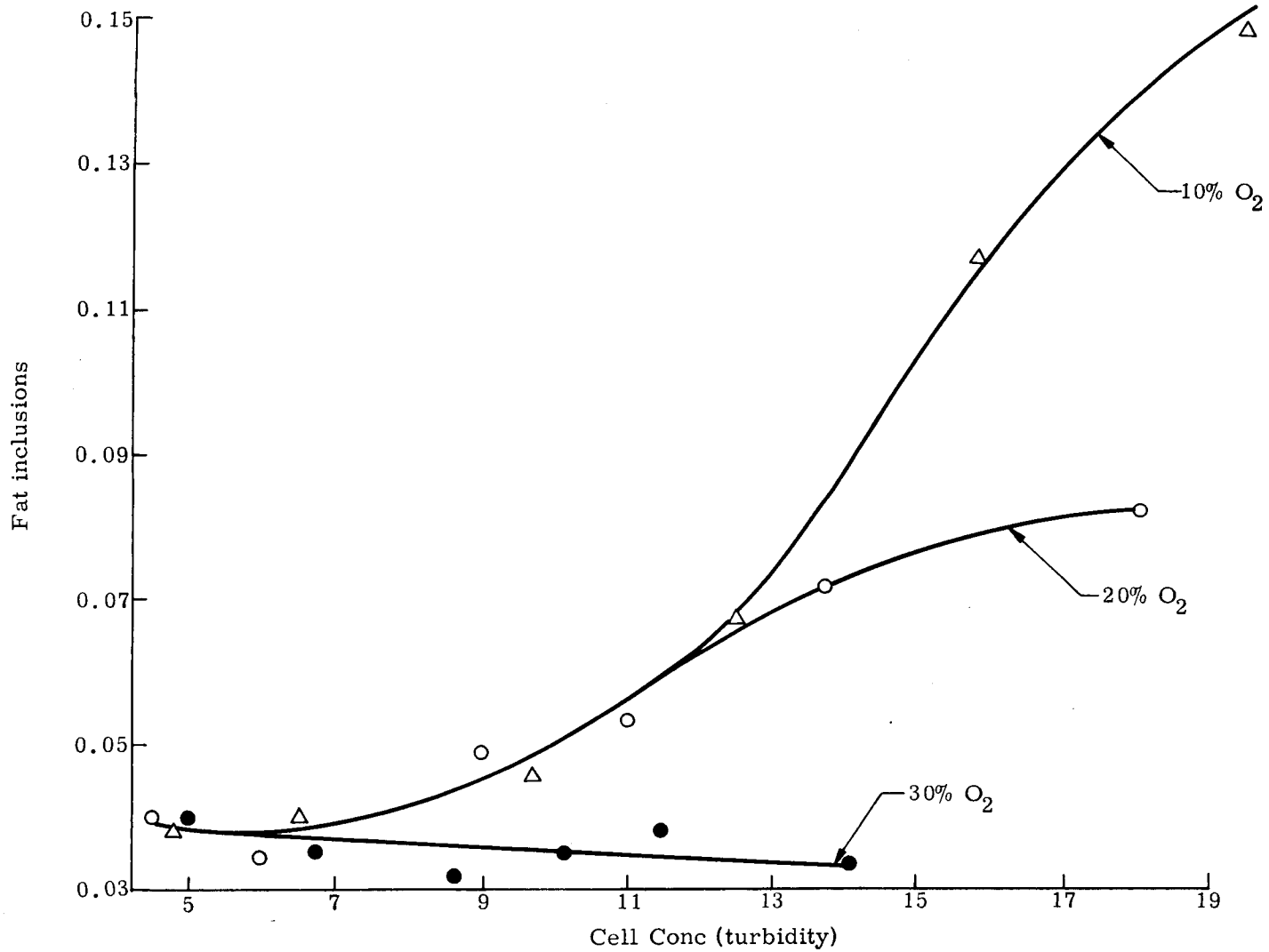


Fig. 2. The Formation of "Fatty" Inclusions as a Function of Oxygen Supply and Cell Concentration
Ordinate: Ratio of OD inclusions over OD cells
Abscissa: Cell concentration in OD units
Conditions as in Fig. 1

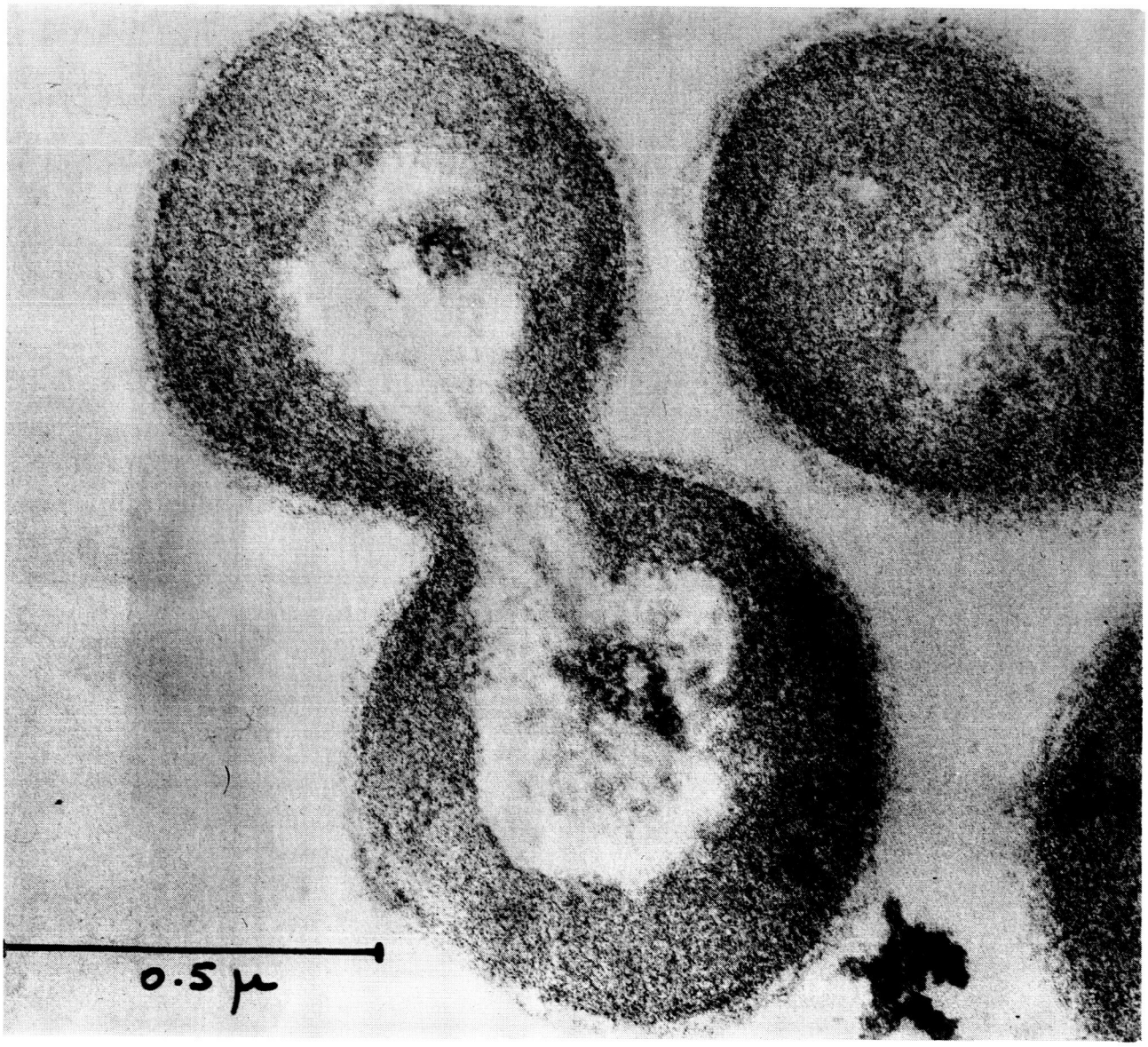


Fig. 3. Electron Micrograph of H Eutropha in Phase of Cell Division

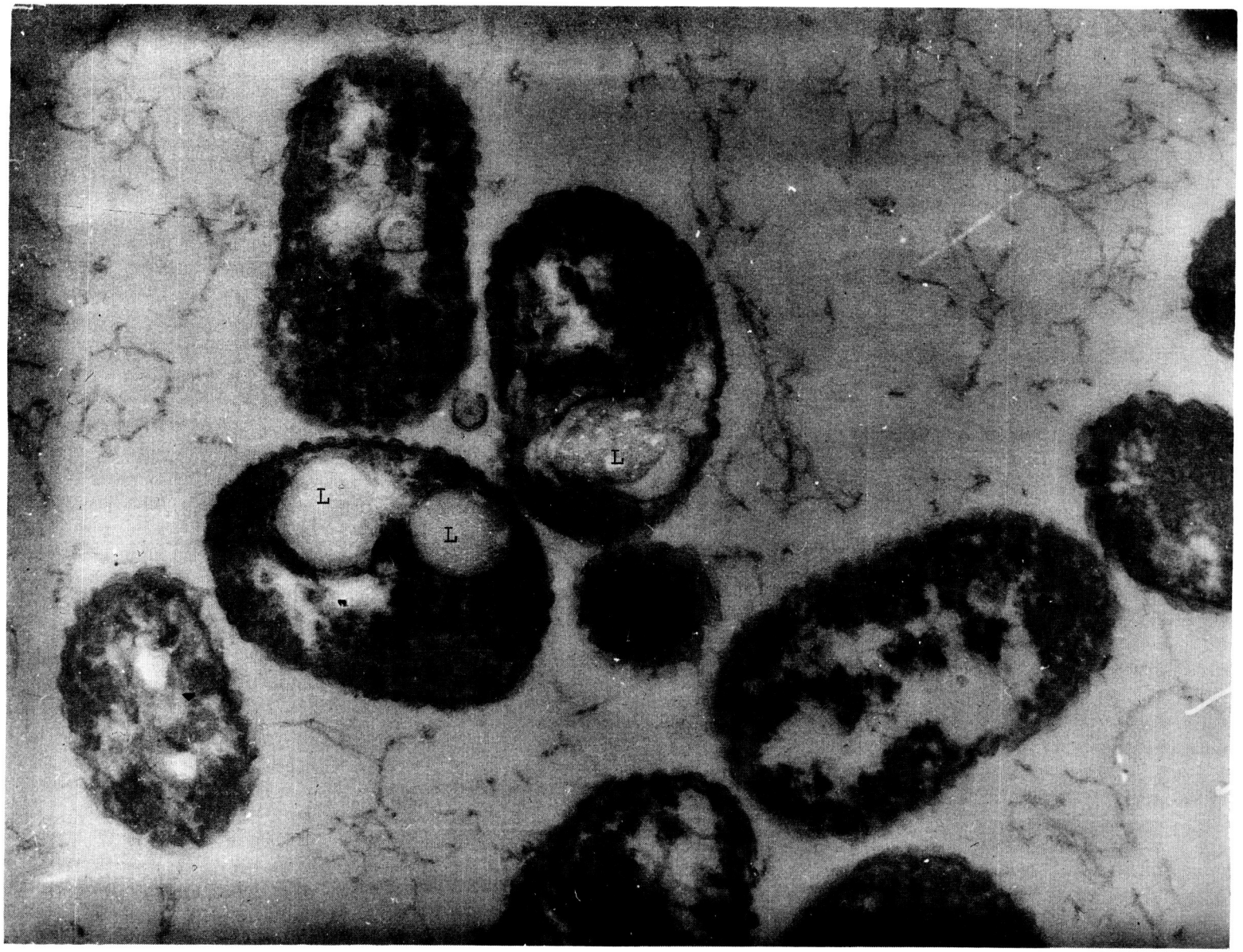


Fig. 4. Electron Micrograph of H Eutropha
L: Fatty inclusion. Average ratio of inclusion was 0.15

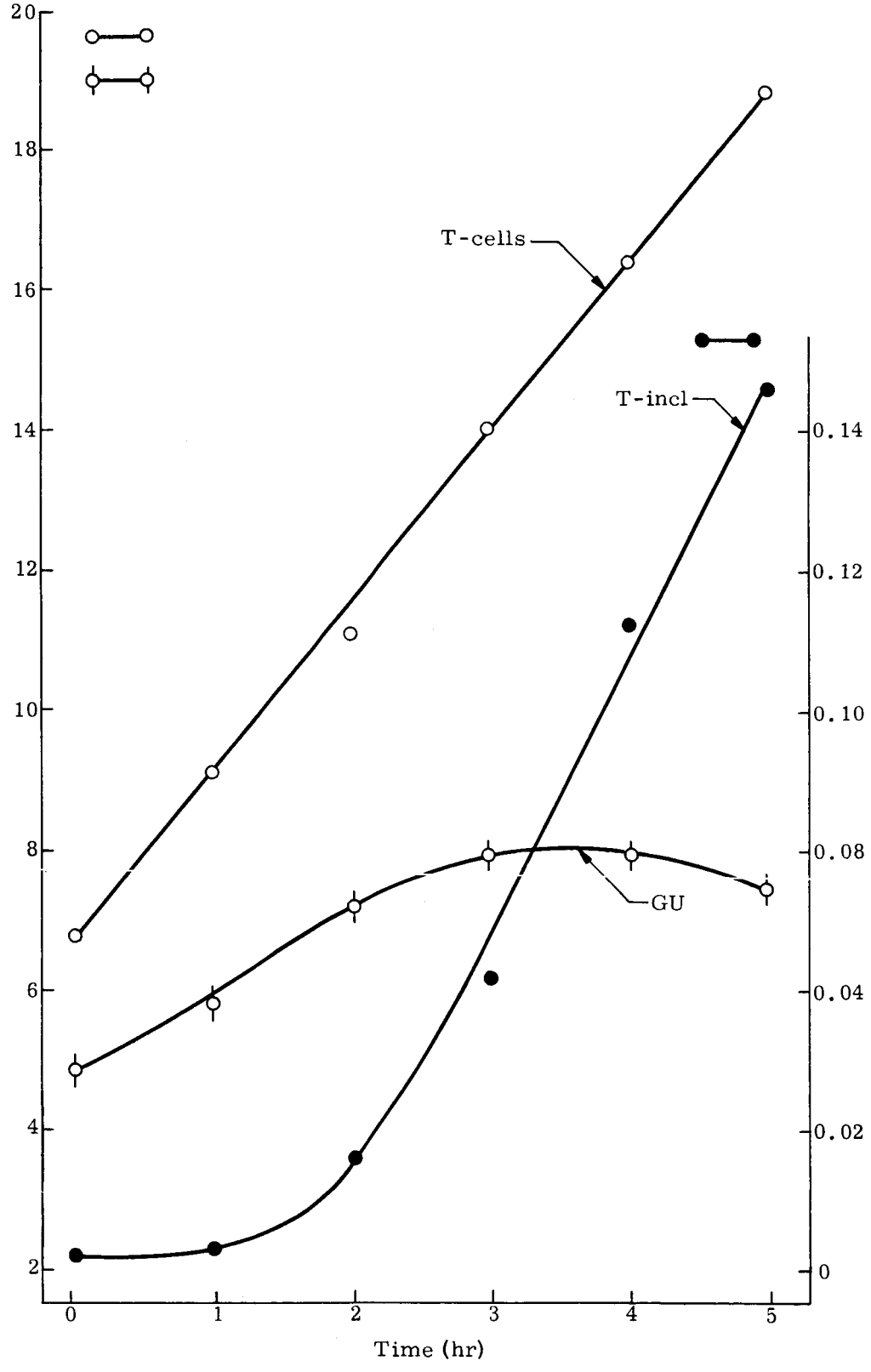


Fig. 5. Time Course Afformation of Cell Material and Fatty Inclusions
Gas phase: 80% H_2, 10% O_2, 10% CO_2

T-cell: Optical density of the suspension
T-incl: Ratio of the optical density of the inclusions over the optical density of cells
GU: Gas uptake per liter suspension per hour

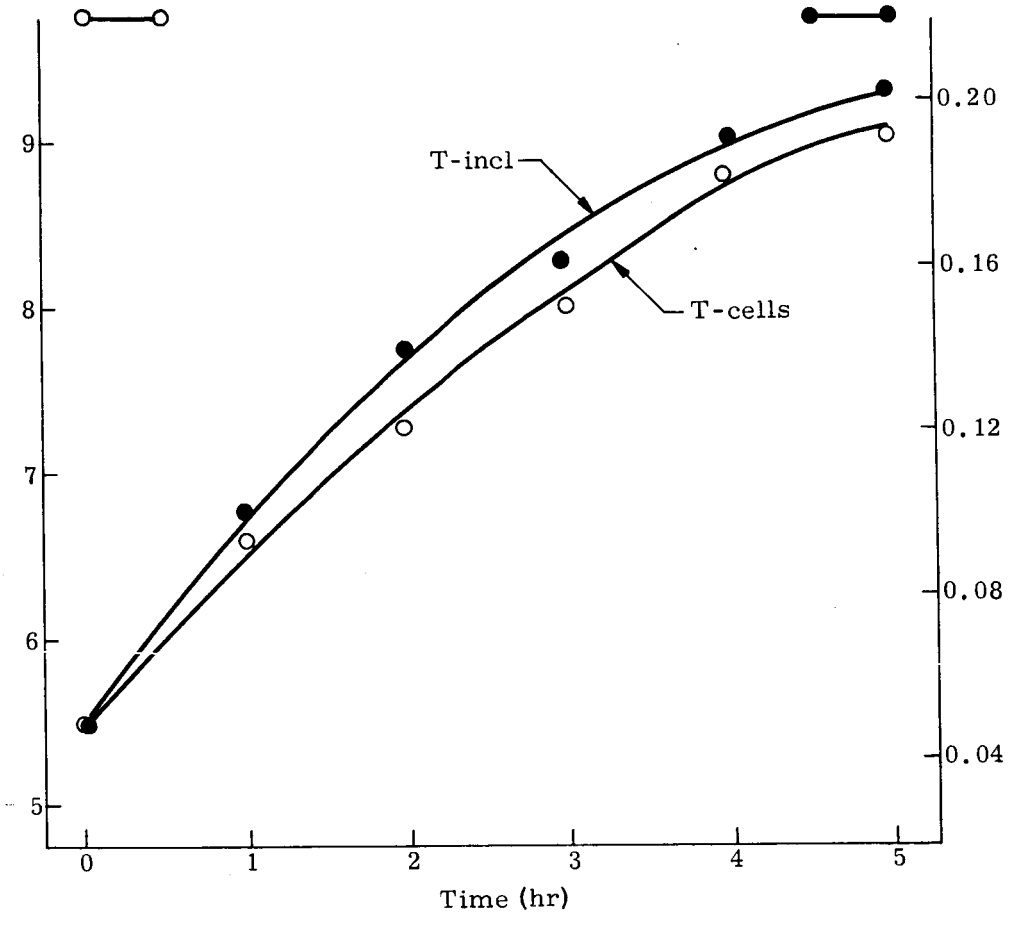


Fig. 6. Time Course of N-Limited Growth
T-cells: Increase in optical density of the suspension (left ordinate)
T-incl: Increase in the ratio of the optical density of the inclusions over the optical density of the cells (right ordinate)

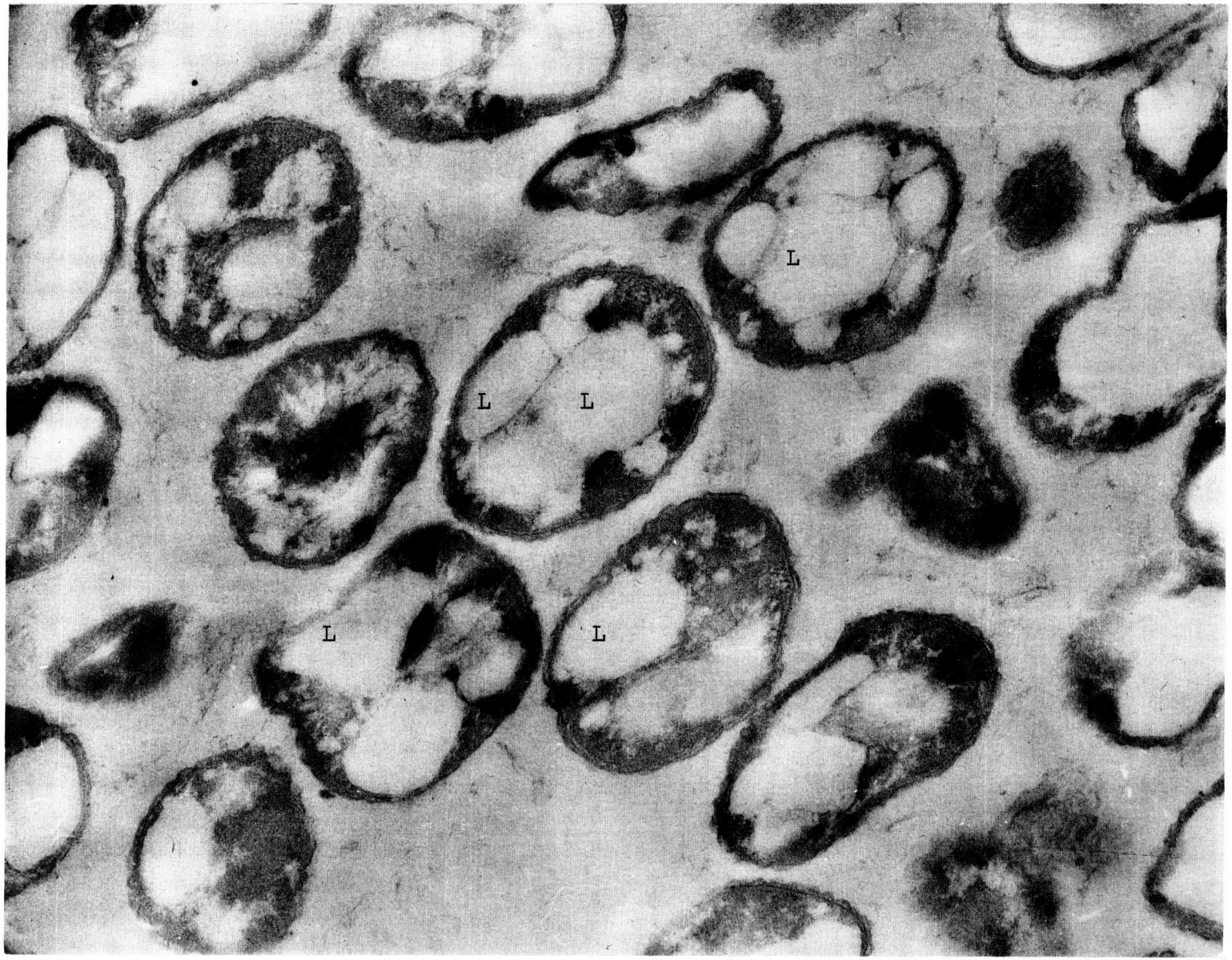


Fig. 7. Electron Micrograph of H Eutropha "Fat" Cells
"L" denotes the fatty inclusions. Cells are nitrogen starved. Average ratio of inclusions was 0.42 (see text).